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TRANSACTIONS

Biochemistry of Filamentous Fungi. V.

Mycelial Constituents of *Oospora sulphurea-ochracea*. Part II.

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In the previous communication⁽¹⁾ the writer recorded the isolation from the dried mycelium of *Oospora sulphurea-ochracea* of crystalline substances, A, B, C and D, with ether extraction.

Of these substances, B, which is obtained in the largest amount, was further investigated for its molecular structure and it was revealed that B contains an atomic arrangement closely related to xanthone.

The molecular formula of B, $C_{10}H_{10}O_4$, given in the previous paper⁽¹⁾ should be at least doubled according to its degradation reactions mentioned below. B melts with decomposition at 257° and is weakly laevo-rotatory. It has two methoxyl and four hydroxyl groups on the basis of $C_{20}H_{20}O_8$. By potash fusion it gave rise to a substance, provisionally marked B', which is to be regarded as a xanthone derivative. This substance B' has two hydroxyl, but no methoxyl. When submitted to zinc-dust distillation, B' was reduced to a substance B'' which is supposed to be a methylxanthene and which can be oxidized again to a corresponding methylxanthone B'''. Of the four possible isomers of monomethylxanthenes only one, 3-methylxanthene, is recorded in the literature which melts at 121° , somewhat higher than B'' (m.p. 113°). As for monomethylxanthenes records in the literature are as shown below and if concluded by comparing their melting points alone, B''' (m.p. 92°) might be either 1- or 3-methylxanthone. The exact position of the methyl group, however, has to be decided by synthesis.

Substance	M.P.	Crystal Form	Fluorescence in conc. H_2SO_4
3-methylxanthene ⁽²⁾	121°	leaflets <i>ex</i> MeOH	—
2-methylxanthone ⁽³⁾	121°	needles <i>ex</i> ligroin	green
1- or 3-methylxanthone ⁽³⁾ or a mixture of them	89~91°	radially grouped, leaflets <i>ex</i> ligroin	—
4-methylxanthone ⁽³⁾	126°	needles <i>ex</i> ligroin	bluish green
3-methylxanthone ⁽²⁾	98°	needles <i>ex</i> EtOH	—

The analytical figures of B'' and B''' also agree in some measure with those of dimethylxanthene and -xanthone, but all the dimethyl derivatives of known constitution given in the literature have much higher melting point than that of B'' and B''', thus the possibility of their identity being precluded.

If it is assumed that B'' is a monomethylxanthene, B''' a corresponding monomethylxanthone and B and B' contain a methylxanthone group as well, the formulae of the latter two will be expanded to $\text{C}_{13}\text{H}_2\text{O}_2 \cdot \text{CH}_3 \cdot (\text{OH})_4 \cdot (\text{OCH}_3)_2\text{C}_4\text{H}_5$ and $\text{C}_{13}\text{H}_4\text{O}_2 \cdot \text{CH}_3 \cdot (\text{OH})_2\text{CHO}_2$ respectively, the elucidation of which will be the subject of future investigation.

To the substance A the formula, $\text{C}_{17}\text{H}_{16}\text{O}_8$, was assigned in the previous paper⁽¹⁾ and the data of acetyl estimation nearly correspond to it. But methoxyl estimation favours $\text{C}_{19}\text{H}_{18}\text{O}_9$ and the selection between these alternatives remains to be decided. By potash fusion of A a phenolic acid A', $\text{C}_{15}\text{H}_{14}\text{O}_8$, was obtained which has no methoxyl.

As to the method of extraction of dried mycelium still another process was resorted to which consists in using first light petroleum for the purpose of removing most of the fat and sterols and then directly acetone without using ether. The extracted matter in acetone was found different in composition from that in the ether method. On the fractional precipitation of acetone extract by evaporating down the solvent, the first fraction consists chiefly of a fresh crystalline substance, differing from any of the above mentioned compounds. It was marked provisionally F. (The substance of m.p. 147° in the previous paper⁽¹⁾ was analysed and as it was found to be a new compound the letter E was assigned to it.) F is a phenolic acid of molecular formula, $\text{C}_{16}\text{H}_{14}\text{O}_8$, having one methoxyl group. The FeCl_3 reaction of its solution is violet, resembling that of A. Potash fusion of F again gave a phenolic acid F' and this was proved to be no other substance than A', by elementary analysis and by melting-point determination alone or mixed with A'. The second fraction of acetone extract consists chiefly of A mixed with a minute amount of B, and the third and last fraction is a dark-coloured, sticky, fatty-smelling magma, from which an inconsiderable quantity of A could be isolated.

On purification of crude F by treating with alcohol and water, another crystalline substance was isolated from the mother liquor which, though not yet analysed, does not agree in m.p. and other properties with any of the substance so far described. The letter G was provisionally affixed to it.

While the substance B was the main product in the case of ether extraction (ca. 6% of dried mycelium), in the acetone process the yield of B diminished to an almost negligible quantity and a fresh substance F appeared instead (ca. 3% of dried mycelium). This difference might be partly due to high temperature to which the extractives in the latter mode of extraction was exposed for a long time (2-3 working days) in the solvent flask of a large Soxhlet apparatus. When a Soxhlet apparatus of ordinary size was employed with a small amount of dried mycelium (25 g. or so), B was usually obtained instead of F even in light petroleum-acetone method. Be that as it may, these facts indicate that there may be some constitutional relationship between B and F and consequently between B and A with F' (=A') as an intermediary.

The light petroleum extract yielded, on evaporation, first sterols which gave strong ergosterol reaction and then fatty matter.

EXPERIMENTAL.

The substance B.

M.p. 257° (decomp.). Laevo-rotatory ($[\alpha]_D^{22} = -4.88$). It contains two methoxyl groups (Found: 15.84%. 2(OCH₃) in C₂₀H₂₀O₈ requires 15.98%).

B is stable to acids but unstable to alkalis; its alkaline solution turns on standing from light yellow to dark brown and on acidifying precipitates a small quantity of yellow substance but the main portion remains dissolved. Ether extracts from the solution yellow sticky matter. With conc. H₂SO₄, it gives orange-yellow solution with green fluorescence, the colour of which grows on standing redder and deeper, the fluorescence fainter, and on addition of a few drops of water the colour is instantly discharged.

Acetylation of B.

B (1 g.) was suspended in an excess of acetic anhydride and a drop of conc. H₂SO₄ was added. Dissolution occurred immediately into a brown solution which was poured into ice-water. Colourless crystals separated which were recrystallized from absolute alcohol. Rhombic platelets (0.8 g.), m.p. 164°, sintering at 160°. (Found: C, 59.98; H, 5.22%. C₂₈H₂₈O₁₂ requires C, 60.43; H, 5.04%. Acetyl estimation (Freudenberg's method). Found: 30.16%. 4 (CH₃CO) requires 30.94%).

Potash fusion of B.

B was submitted to potash fusion with ten times its weight of KOH

and a small quantity of water at $200\sim 230^{\circ}$ in a metal bath. The product was dissolved in H_2O and the solution saturated with CO_2 . No precipitation occurred and nothing could be extracted from the solution with ether. The solution was then acidified with H_2SO_4 and as there was no precipitation the solution was shaken several times with ether. The combined extracts were dried with anhydrous Na_2SO_4 and the solvent was evaporated off to dryness. Oily residue solidified on standing to radially grouped needles. It is insoluble in benzene, chloroform and light petroleum, readily soluble in ethyl acetate, ethyl or methyl alcohol, moderately soluble in ether and water. It dissolves in $NaHCO_3$ solution with effervescence. The $FeCl_3$ reaction is brown. By adding light petroleum to its ether solution it precipitates as slender needles stuck on the wall of the vessel. It is exceedingly hygroscopic and when taken out of the vessel, by absorbing moisture it is quickly converted to a yellow substance which is no longer soluble in ether. The oily residue therefore was boiled with water under reflux for a few hours. Yellow crystals separated abundantly in the meantime. Yield, ca. 70% of B used. This is marked B'. For purification it was dissolved in alcohol and precipitated with the addition of water. M.p. 290° , optically inactive. (Loss of weight on drying in vac. at 120° : 5.90, 5.76%. Found: in anhydrous substance, C, 63.06, 62.64; H, 3.68, 3.82%. $C_{15}H_{10}O_6 \cdot H_2O$ requires H_2O , 5.92%, and $C_{15}H_{10}O_6$ requires C, 62.94; H, 3.50%). Molecular weight determination by Rast's camphor method and Pirsch's pinene dibromide method were neither successful owing to its insolubility in these substances.

It is insoluble in H_2O , ether, light petroleum, benzene and chloroform, soluble in ethyl or methyl alcohol and acetone. It is stable to conc. H_2SO_4 , dissolves in it, giving yellow solution with green fluorescence from which B' is recovered unchanged on dilution with water.

Acetylation of B'.

B' (1.0 g.) was suspended in an excess of acetic anhydride and a drop of conc. H_2SO_4 was added to it. Reaction occurred immediately giving a brown solution, which was poured into ice-water. Separated solids were recrystallized from alcohol, pale yellow thin platelets were obtained. It melts at 186° , sintering at 180° . It is insoluble in cold $NaOH$. Its alcoholic solution gives no reaction with $FeCl_3$. Its analytical figures nearly correspond to diacetyl derivative. (Found: C, 61.09; H, 4.15%. Acetyl estimation (Freudenberg's method): 24.16%. $C_{15}H_{10}O_6 \cdot (CO.CH_3)_2$ requires C, 61.62; H, 3.78%. $2(CH_3CO)$, 23.24%).

Zinc dust distillation of B'.

B' (2.0 g.) was mixed with twenty times its weight of zinc dust and

submitted to distillation in a stream of hydrogen, temperature being kept below red heat. Colourless crystalline mass was deposited on the cool part of the tube (0.5 g.). When the temperature was higher, the distillate was red-tinted, its amount less, and the gas escaping from the tube had strong benzene-like odour. In the case of lower temperature the escaping gas was of faint, phenolic smell.

The deposited mass was dissolved in ether in which it is readily soluble, giving a yellow solution with green fluorescence. In case the distillate was heavily red-tinted, the solution was reddish and of intense green-yellow fluorescence, resembling an eosin solution. In any case by shaking with animal charcoal the filtrate lost both colour and fluorescence. Ether was then driven off and the residue was recrystallized from alcohol. Thin lamellar crystals, m.p. 113°, were obtained (0.15 g.) (B''). Its melting point and appearance resemble those of fluorene but analytical figures indicate that this is not the case. (Found: C, 85.64, 85.45; H, 6.32, 6.68%. Mol. wt. (cryoscopic in benzene), 182.9. $C_{14}H_{12}O$ requires C, 85.71; H, 6.12%; mol. wt., 196. $C_{15}H_{14}O$ requires C, 85.71; H, 6.67%; mol. wt., 224).

The conc. H_2SO_4 solution of B'' is yellow and fluoresces intensely green (fluorene shows blue fluorescence in conc. H_2SO_4 solution).

Oxidation of B''.

B'' (0.3 g.) was dissolved in glacial acetic acid and CrO_3 (0.3 g.) dissolved in a small quantity of water was added to it. The solution was warmed till it turned green, poured into water and extracted with ether. The residue of the ether extract was taken in MeOH and H_2O was added to it till it became turbid. After standing overnight in ice the separated solids were collected (0.2 g.), which were recrystallized from ligroin (B'''). Needles, m.p. 92°. (Found: C, 79.77; H, 5.18%. $C_{14}H_{10}O_2$ requires C, 80.00; H, 4.76%. $C_{15}H_{12}O_2$ requires C, 80.36; H, 5.36%). The solution of B''' in conc. H_2SO_4 displays an intense blue fluorescence, quite similar to that of xanthone.

The substance A.

M.p. 214°, optically inactive. It is slightly soluble in hot water (in the previous paper⁽¹⁾ it was described insoluble in water, which should be corrected). (Zeisel. Found: 15.85, 15.87%. 2 (CH_3O) in $C_{19}H_{18}O_9$ requires 15.90%; in $C_{17}H_{16}O_8$ requires 17.82%).

Acetylation of A.

A was suspended in an excess of acetic anhydride and a drop of conc. H_2SO_4 was added. The resulted solution was poured into water. The separated solids were well washed, dissolved in MeOH and precipitated by the addition of H_2O . Colourless needles, m.p. 147°. The alcoholic solution of the acetate

gives no colour with FeCl_3 . (Acetyl estimation. Found: 19.68, 20.30%. $2 (\text{CH}_3\text{CO})$ in $\text{C}_{19}\text{H}_{16}\text{O}_9$. $(\text{CH}_3\text{CO})_2$ requires 18.14%; in $\text{C}_{17}\text{H}_{14}\text{O}_8 (\text{CH}_3\text{CO})_2$ requires 19.91%).

Potash fusion of A.

A was fused with ten times its weight of KOH and a small quantity of water at 250° . The product was dissolved in water and saturated with CO_2 . Nothing separated. The solution was next acidified with H_2SO_4 . Precipitated solids (A') were collected (ca. 30% of A used) and recrystallized from much water using animal charcoal. Colourless needles, m.p. (decomp.) 233° . (Loss of weight on drying in vac. at 120° : 5.30%. Mol. wt. (cryoscopic in camphor), 312.5. Found: in anhydrous substance, C, 56.03, 55.96; H, 4.25, 4.50%. $\text{C}_{15}\text{H}_{14}\text{O}_8 \cdot \text{H}_2\text{O}$ requires H_2O , 5.29%; mol. wt., 340; and $\text{C}_{15}\text{H}_{14}\text{O}_8$ requires C, 55.90; H, 4.35%.) It is readily soluble in EtOH, moderately soluble in ether, sparingly soluble in cold, more soluble in hot water. The FeCl_3 reaction of its solution is purple.

The substance E.

This was isolated in one case from the Fraction I of the ether extraction method, as described in Part I. Pale yellow block crystals of characteristic angular form, with one molecule of water of crystallization. (Found: C, 58.86; H, 4.74%; loss of wt. in vac. at 100° , 5.48%; in anhydrous substance, C, 61.78; H, 4.48%. $\text{C}_{17}\text{H}_{14}\text{O}_7 \cdot \text{H}_2\text{O}$ requires C, 58.62; 4.60; H_2O , 5.17%, and $\text{C}_{17}\text{H}_{14}\text{O}_7$ requires C, 61.82; H, 4.24%.) It is insoluble in NaHCO_3 , soluble in NaOH. With FeCl_3 it gives no reaction.

The substance F.

This was isolated from the first fraction of acetone extract as described above. When recrystallized from alcohol and water, F separated out in fibrous, flocky precipitate resembling finely torn absorbent cotton.

F crystallizes in colourless glistening thin needles, m.p. (decomp.) 239° . It is readily soluble in alcohol, soluble in hot water from which on cooling it separates out with one molecule of water of crystallization. (Found: in anhydrous substance, C, 57.15, 57.41; H, 4.40, 4.56%. $\text{C}_{16}\text{H}_{14}\text{O}_8$ requires C, 57.49; H, 4.19%). On recrystallization from glacial acetic acid it separates out in thin needles of silky lustre with one molecule of solvent of crystallization, which is lost on heating in vac. over 100° for a few hours. (Found: C, 54.86; H, 4.90; loss of wt. on heating, 15.63, 15.63%. $\text{C}_{16}\text{H}_{14}\text{O}_8 \cdot \text{C}_2\text{H}_4\text{O}_2$ requires C, 54.82; H, 4.57; $\text{C}_2\text{H}_4\text{O}_2$, 15.23%).

Potash fusion of F.

F (1 g.) was fused with KOH (10 g.) at 220° . The product was dissolved

in water and saturated with CO_2 . No precipitation and nothing extracted with ether. On acidification with H_2SO_4 , abundant quantity of needle crystals separated out (0.6 g.) (F'). The crude material was dissolved in acetone in which it is readily soluble, treated with animal charcoal, and precipitated with H_2O . It may also be recrystallized from hot water. It melts with decomposition at 233° alone or mixed with A', gives the same analytical figures, thus their identity being confirmed. (Loss of wt. on drying, 5.04; in anhydrous substance, C, 55.53; H, 4.33%. $\text{C}_{15}\text{H}_{14}\text{O}_8 \cdot \text{H}_2\text{O}$ requires H_2O , 5.29% and $\text{C}_{15}\text{H}_{14}\text{O}_8$ requires C, 55.90; H, 4.35%).

The substance G.

The mother liquor of the recrystallization of F was concentrated under reduced pressure to a small bulk and the precipitated solids were collected. The crop was yellow-coloured and microscopic examination revealed that it was a mixture of thin white needles and yellow angular thick crystals. It was dissolved in hot water and on cooling yellow thick prisms separated out first which was quickly collected. The filtrate from this fraction on standing gradually became turbid and separated a mixture of F and G from which further crop of G could be obtained by treating with hot water (in all ca. 0.2% of dried mycelium). G crystallizes in pale yellow prisms, m.p. 205° after sintering, soluble in NaHCO_3 and NaOH ; FeCl_3 reaction, purple-brown. Its red solution in conc. H_2SO_4 turns colourless on addition of a drop of water.

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SUMMARY.

(1) The substance B, one of the mycelial constituents of *Oospora sulphurea-ochracea*, was investigated on its structure and as one of breakdown products a methylxanthene was isolated. The structural relation of B to xanthone was suggested.

(2) The structural connection between A and B was discussed basing upon experimental facts.

(3) The isolation of some more crystalline constituents was recorded, thus hitherto isolated crystalline compounds from this mould amounting to 7 in all.

REFERENCES.

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- (3) Ullmann u. Zlokasoff: Ber., **38**, 2114 (1905).

ABSTRACTS

from

TRANSACTIONS published in JAPANESE

(Pages refer to the Japanese originals of this volume unless otherwise noticed)

On the Synthesis of Amine from the Alcohol. (p. 1~5): By
Yuichi SHINOZAKI and Hiraki KUBO. (Central Laboratory, South Manchuria Railway Co.,
Dairen. Received Nov. 7, 1936.)

Über das Eiweisz der gereinigten Panicum Crus-galli, L. var frumentaeum, Hook f. (S. 6~10): Gearbeitet von Tetsujiro OHARA.
(Bei der Provinzial-Landwirtschaftlichen Schule in Morioka. Received Oct. 23, 1936.)

Infolge meiner Untersuchung über die Ernährungsschemie habe ich schon früher darüber einen Bericht erstatten, dasz das Eiweisz des Panicums sowohl an Qualität auch an Quantität gereinigten Reises weitaus übertrifft und die Frucht des Panicums eine grosze Menge Vitamin B (antiberiberisch) enthalten, auch in der gereinigten Frucht desselben genügenden Vitamin B enthalten ist, Beriberi zu heilen und verhüten. Weiter habe ich nun beabsichtigt, die Untersuchungen über die chemische Wirkung der genannten Frucht.

Ausser der Berichten über die allgemeinen Analisisierung der betreffenden Frucht, erstatten von hyg. Laboratorien, von der Sapporo-Landwirtschaftlichen Fakultät und von mir selbst, gibt es nur wenige chemische Untersuchungen darüber, doch betreffs blauen unreifen Frucht dieser Pflanze sind zwei Berichte über die allgemeine Analisisierung und die über das Resultat der Verdauungsprozent bei Ziegen sowie die Berichte über die Kleie dieser Frucht, weiter die über die Zusammensetzung der Kleie der Frucht erstattet. Neuerdings hat Herr Han-emon Ito in seiner Untersuchung über Hiyeol, das öl der Frucht berichtet. Abgesehen von solchen Berichten, hat man weder Untersuchungen über das Eiweisz noch die näheren Untersuchungen über die Asche und Kohlenhydrate der Frucht, und ich habe deshalb über den Eiweisz der Frucht, welche durch Anwendung des Mittels des Zerstampfens, eines selbstdampfenden Mittels (einer Art der Bereitung gekochtes, getrocknetes Reis) zu reinigen ist, untersucht und bin zur folgenden Ergebnissen gelangt.

(1) Nachdem der Eiweisz der Frucht extrahiert, gereinigt, dann die Reaktion des betreffenden Eiweiszstoffes geprüft worden waren, zeigte es sich,

dass die meisten Teile der Farbenreaktion, Fallungsreaktion und der Erstarrungsreaktion positiv bleibe, während nur Molisch's Reaktion negativ sei.

(2) Die gesamte Stickstoffe der gereinigten Frucht des Panicums ist als lufttrockenes 1,95%, während sie als feuertrockene 2,22% enthält und deren Eiweiszstoff in der feuertrockenen 2,15%, d.h. 95,85% sämtlichen Stickstoffe enthält.

(3) Bei der Prüfung der in der gereinigten Frucht des Panicums enthalten Stickstoffverbindungen, deren Arten und Gehalt wurde es herausgestellt, dass in den Eiweiszstoffe der Frucht des Panicums eine beträchtliche Menge der 75%igen alkoholischen Stickstoffe (prolaminartigen Stistoffe) enthält, welche rund 37,1% der gesamten Stickstoff beträgt. Nächstgroszen Gehalt hatte der 0,2%ige NaOH-lösliche Stickstoffe (glübtelinartige Stickstoffe), deren sämtlich Stickstoffe 2,207% beträgt. Der wasserlösliche Stickstoff (albuminartiger Stickstoff) enthält 11,7% der gesamten Stickstoffe, während der in allen Lösungsmitteln unlösliche Stickstoff 31,98% betrug.

Der Grund, weshalb der unlösliche Stickstoff darin so viel enthalten ist, beruht darauf, dass bei der Reinigung zwei Verfahren wie Dampfen und Trocknen angewandt wurden, und daraus lässt sich vermuten, dass der lösliche Stickstoff in den unlösliche verwandelt.

(4) Durch Prüfung der Verteilung der Aminosäure, welche den Eiweiszstoff der Frucht des Panicums bilden, wurde es bekannt gemacht, dass der Gehalt der Diaminosäure verhältnismässig gross sei. Er beträgt zwar 28,2% der gesamten Stickstoffe, worin 11, 95% der amineartige Stickstoff ist, und der nächstgrosze ist der histidinartige Stickstoff, welcher 9,5% enthält; der lysineartige ist aber der 2,07%ige. Der monoaminoartige Stickstoff enthält etwa 50%, worin sich der 12%ige nichtaminoartige und Tryptophan, Prolin, Oxplolin u.a. befinden.

(5) Die obenerwähnten Resultaten zeigen sich sowohl, dass die Qualität des Panicums sehr vortrefflich sei, auch dass der Eiweiszstoff des Panicums gegen Reiseiweisz bei weitem besser sei, wie es in meiner Ernährungsprüfung bestätigt wurde, und das kann auch wohl genug sein, es fast zu beweisen, dass der Eiweisz des Panicums als Ersatz Reiseiweisz einen bedeutenden Wert habe.

Studies on High Pressure Hydrogenation of the Castor oil.

I. On the Reduction of the hydroxy group. (pp. 12~17); II. On the Formation of Octadecandiol (1, 12) and the Capillary Chemical Properties of the Sodium Salt of Octadecandiol-disulphonic acid-ester. (pp. 18~22): By Yuichi

SHINOZAKI and Sanroku KAGAWA. (Central Laboratory, South Manchuria Railway Co., Dairen, Received Oct. 12, 1936.)

Enzyme Chemical Investigation of Formosan Snake Venoms, V. Studies on the Activation of Peptidase by the Snake Venoms.
Part VIII. On the Active Constituent in the Venoms of Taiwanhabu [*Trimeresurus mucrosquamatus* (CANTOR)] and Taiwankobura [*Naja naja atra* (CANTOR)], for the Activation of Dipeptidase. (pp. 23~34): By Yoshio TSUCHIYA. (Laboratory of Nutrition Chemistry, Faculty of Science and Agriculture, Taihoku Imperial University, Japan, Received Nov. 10, 1936.)

On the Koji-amylase Part V.—The Difference between the Amount of Extraction of Koji-amylase, Maltase, Saccharase and Protease with Salt Solution. (pp. 35~40): By Yuzo TOKUOKA. (Tsunekichi Okura Brewery, Received Nov. 10, 1936.)

As was already reported in the previous papers (Part I~IV), neutral salts revealed a remarkable effect on the extraction of koji-amylase adsorbed by starch of the steamed rice, however koji-maltase would be easily extracted with distilled water.

In the present paper the action of the various kinds of enzyme preparations were compared in order to ascertain whether salt solution would reveal any noticeable effect on the extraction of koji-maltase, saccharase or protease.

For the enzyme preparations alcohol was used for the precipitation of the solution which were obtained in the following manners:

(a) Saké-koji was extracted with water, (b) Saké-koji was extracted with 1% NaCl solution, (c) Saké-koji was thoroughly washed with cold water and then extracted with salt solution (see Part IV), (d) After being washed several times with distilled water, saké-koji was kept for 24hrs at 30° in the presence of toluol and chloroform in order to cause autolysis. The autolysed residue was washed thoroughly with distilled water, and then extracted with salt solution.

The action of salt solution on the extraction of koji-enzymes was found to be very peculiar to amylase, although with the other three enzyme, a considerable increase (ca 20%) in the amount of extraction was again found in the salt extract (b) when these were compared in the water extract (a).

It is probable that the other three enzymes would partly exist in the state of endo-enzyme or desmo-enzyme which might be extracted with salt solution.

The amount of extraction of saccharase and protease was found to be increased by autolysis, however the amount of maltase was not altered when compared (c) & (d) preparations.

As was pointed out in the previous paper with maltase (part IV), the amylase preparation (d) was again found to contain nearly 1% of each of saccharase and protease when compared with the preparation (a).

The preparation (d) contained nearly 10% of each of the other three enzymes when compared with the preparation (b) which would be taken as a standard of koji-diastase preparation.

The Manurial Effect of Guano on Rice-plants. (pp. 41~45): By Chikafumi ICHIKAWA. (The Soma Agricultural and Sericultural School, Received Oct. 20, 1936.)

The manurial effect of Guano was determined by culturing rice plant in pots and was compared with that of ammonium-sulphate. Guano was utilized 51.98% and ammonium sulphate 58.99, so taking the latter 100, Guano was utilized about 90%. Accordingly Guano was found inferior to ammonium sulphate slightly.

The Influence of Monochromatic Lights on the Action of Soya-Urease. (pp. 46~51): By Reitaro MURAKAMI. (Agricultural College, Utsunomiya, Received July 31, 1936.)

In order to investigate the influence of spectral monochromatic lights on the action of soya-urease prepared from japanese yellow soyabean, the author has added 5 cc of 0.5% enzyme solution into the bottles containing 50 cc of 1% urea solution and were connected the absorption bottles containing sulphuric acid.

The bottles were placed within the tinned boxes, at the front windows of which the monochromatic filters of red, yellow, green, blue, violet and ultra vit glass were placed. The filters of white and black passing respectively all or no visible rays were also applied as the controls. The boxes containing the bottles were incubated at 37°C and lighted by an electric lamp through the layers of copper sulphate solution and the filters.

The bottles were taken out from each box at intervals of 2, 4, 6, 8, 10

and 14 hours after the incubation and the end product, ammonia were determined by Kjeldahl method.

In this experiment, the apparent effect on the soya-urease was found to be strongest under the rays transmitted through the ultravit glass and stronger under the rays of white or yellow lights. It was weaker under the rays of violet than the rays of red, green and blue. The action was almost inferior in the dark.

But the proportions of the ammonia produced by the enzyme for an unit intensity of the transmission rays through each filter was found to be in inverse effect in comparison with their intensities.

Hence, the rays present the positive stimulant for the soya-urease but the effect has been decreased successively with their intensities.

Über die technische Citronensäuregärung I. Mitteilung. (S. 52~62): Von R. NAKAZAWA, Y. TAKEDA und M. NAKANO. (The Department of Industry, Government Reserch Institute, Taiwan, Japan, Received Nov. 17, 1936.)

Résumé

Der hier von Awamori-Kôdi aus Okinawa Provinz isolierte Schwarz-Aspergillus hat die Tätigkeit der technische Citronensäurebildung aus Rohrzucker in der künstliche Lösung gezeigt. Die Lösung enthält Rohrzucker 10%, Na-Nitrat 0.3%, K-Biphosphat 0.03%, Mg-Sulfat 0.01% und Ca-Carbonat 1.0% in Leitungswasser, wobei seine Wasserstoffionenkonzentration wurde mit dem Zusatz von decinormale Salzsäure in die Grenze pH 4.4~4.6 gepuffert. In Versuchen mit 50 cc Lösung die konstante und höchste Ernte an krystalle Citronensäure betrug etwa 65% des angewendeten Rohrzuckers. Die Oxalsäurebildung, welche ist eine merkmale Character dieser Arten, wurde hierbei völlig zurückgehalten in der Lösung. Dieser Pilz ist ein neue Variant des Asp. Awamori Nakazawa, der von Autoren in 1918 aus Awamori-Kôdi isoliert worden war, und über seine mycologische Beschreibung wurde in andere Bericht dieses Laboratoriums mitgeteilt als Asp. Awamori var. fumeus nov. var.

Diese Arbeiten sind von der Auftrage Herrn Takeda-Tyôbei zu Osaka durchgeführt worden.

Über die verschiedene Eigenschaften von Aspergillusarten (III). (S. 63~70): Von Y. TAKEDA und O. TAKEUTI. (The Department of Indust-

ry, Government Research Institute, Taiwan, Japan, Received Nov. 4, 1936.)

Biochemical Studies on Glutathione. Report I.—Some correlations between glutathione contents of arterial blood and of venous blood of normal Rabbits. (pp. 71~79): By Masayoshi OGAWA. (Dept. of Nutrition Tokyo Municipal Hyg. Lab, Received Oct. 27, 1936.)

Glutathione has recently received considerable attentions as an active reducing substance in living tissue, but little is known about reduction and oxidation of the substance of blood yet. Therefore the author tried to determine oxidised (GS-SG) and reduced (G-SH) form of that substance of arterial and of venous blood by the method of Prof. Okuda and the author, employing ten heads of normal rabbits, and obtained the following results.

Glutathione contents of whole blood seems to differ with each individuality in some respects, but there observed some correlations between reduced, oxidised, and total glutathione contents of arterial and of venous blood with every individual. 100 g of whole arterial blood of normal rabbits contains 19.66~46.47, av. 31.37 mg of GSH, 17.09~29.82, av. 22.31 mg of GS-SG, and 36.86~65.18, av. 53.68 mg of total glutathione, yet venous blood contains 26.93~51.82, av. 36.53 mg of GSH, only 4.06~17.72, av. 9.88 mg of GS-SG, and 36.27~59.95, av. 46.41 mg of total glutathione. Then glutathion which seems to be concerned with oxidation and reduction (GS-SG→GSH) system in blood circulation is shown in the following table.

	GS-SG, seems to be reduced to GSH.	Glutathione, seems to be consumed.	GS-SG, seems to be concerned with GS-SG→GSH system.
	36.53 (GSH contents of venous blood)–31.37 (GSH contents of arterial blood.)	53.68 (total glutathione contents of arterial blood)–46.41 (total glutathione contents of venous blood.)	22.31 (GS-SG contents of arterial blood)–9.88 (GS-SG contents of venous blood), or the sum of the above mentioned, (5.16+7.27)
mg per 100 g blood.	5.16	7.27	12.43
per total glutathione contents of arterial blood.	9.69%	13.54%	23.21%

From the above facts the author concluded that all the glutathione in arterial blood is not seems to be concerned with oxidation and reduction system (GS-SG→GSH) except only 23.21% of them at the same time, and then 13.54% of total glutathione in arterial blood seems to be consumed du-

ring the course of blood circulation.

I wish to thank Dr. Y. Fujimaki for his kind interest in this work.

Biochemical Studies on Glutathione. Report II.—Effect of Ultra-violet ray radiation on blood glutathione. (pp. 80~88): By Masayoshi OGAWA. (Dept. of Nutrition Tokyo Municipal Hyg. Lab., Received Oct. 27, 1936.)

In the previous communication the author reported some correlations between the glutathione contents of arterial and of venous blood of normal rabbits by the method of Prof. Okuda and the author.

In the present report the author investigated the effects of Ultra-violet ray radiation on oxidised (GS-SG) and reduced (GSH) glutathione contents of blood (of arterial and of venous) employing 6 heads of normal rabbits. These animals were phlebotomised at intervals after exposed to Ultra-violet ray radiation from full quartz mercury arc (Acme Laboratory Arc 6 amp. 110 volts. wave-length 2000 3300 Å) at a distance of 20 cm for 10 minutes.

The following table shows the results obtained;

mg per 100 g blood	arterial blood			venous blood		
	GSH	GS-SG	Total	GSH	GS-SG	Total
before radiation	34.15	23.94	58.09	38.54	10.75	49.24
immediatly after radiation	34.66	9.38	44.03	32.08	12.86	44.94
in 30 min~1 hour after radiation	33.24	44.75	77.99	42.85	26.41	69.26
in 2.5 hours after radiation	50.81	29.58	80.39	47.08	21.26	68.34
in 4.5~5 hours after radiation	35.38	24.55	60.33	34.58	19.43	54.01
in 20 hours after radiation	35.31	24.33	59.64	35.66	16.13	52.02

The results show that Ultra-violet ray radiation was found to produce a conspicuous effect on glutathione contents of blood, but the normal condition appears to be restored in 4.5~5 hours after the radiation.

Reduced glutathione (GSH) contents of blood is extremely decreased immediately after the radiation, but in 30 minutes~1 hours it is increased highly. It is interesting that oxidised glutathione (GS-SG) contents of venous blood immediately after the radiation is conspicuously increased, and yet of arterial blood it is then decreased on the contrary, though in 30 minutes~1 hour it is highly increased.

But the explanation in these lines must await further experiment.

I am deeply indebted to Dr. Y. Fujimaki for his interest in this work.